Bioaccumulation of Chemical Contaminants in Transplanted and Wild Mussels in the Duwamish River Estuary, Puget Sound, Washington

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Introduction

This paper presents some of the results of a year-long monitoring program aimed at better understanding chemical contamination in the Duwamish River estuary, part of the highly urbanized Green/Duwamish River watershed in western Washington. Chemicals enter the lower watershed both from point sources—such as permitted industrial discharges, treatment plants, storm water, combined sewer overflows (CSOs), and accidental spills and leaks—and from nonpoint sources—including runoff, atmospheric deposition, and ground water. Of particular interest in this study are the chemical contributions of CSOs relative to other sources of pollution entering the Duwamish River estuary.

These studies were conducted in September 1996 (dry season) and in March 1997 (wet season) employing both transplanted and wild mussels. Analysis of the concentration of chemicals in the soft tissues of mussels was the basis of chemical monitoring. It is well established that tissue contamination in mussels and other bivalves increases or decreases as environmental contamination increases or decreases (NOAA, 1989). It is for this reason that mussels are particularly good sentinels of contamination.

The key questions addressed by this research included: 1) Are chemicals entering the Duwamish River estuary bioavailable and bioaccumulated? 2) Do concentrations of bioavailable chemicals change seasonally? 3) Are the contributions of chemicals from CSOs measurable in a biological receptor, e.g., mussels? While mussel tissue was analyzed for a wide variety of metals and semivolatile (extractable) organic compounds, only the results of analyses for copper, tributyltin (TBT), polychlorinated biphenyls (PCBs), and lipids are reported in this communication.

Methodology

Mussel Deployment and Retrieval

Juvenile mussels, *Mytilus galloprovincialis*, were collected from the Taylor United Inc. mussel farm on Totten Inlet, Washington. Following the general recommendations of Salazar and Salazar (1995), 50 mussels 25–40 millimeters (mm) in size were loaded into individually compartmentalized mesh bags using plastic cable ties. Plastic oyster cultch netting (15-mm mesh stretch) was used for this purpose. Prior to being loaded into mussel bags, all mussels were weighed to the nearest 0.001 gram (g). Statistical analyses (see methodology described below) determined that mussel lengths and weights in all groups were the same ($P<0.05$) at the time of deployment.

The mussels were deployed at each site by boat. Ten mesh bags containing 50 mussels each were suspended from a float anchored at each study site. Five mesh bags were suspended 1 meter (m) below mean lower low water (MLLW); five mesh bags were suspended 3 m below MLLW but at least 1 m above the bottom. The mesh bags were deployed for four weeks both in September 1996 (dry season) and in March 1997 (wet season).

Wild mussels, *Mytilus trossulus*, were collected coincident with retrieval of transplanted mussels. Wild mussels were collected randomly from concrete pilings 1 m above MLLW at some of the mussel transplant locations. Fifty mussels 25 to 40 mm in size comprised each wild mussel sample. No less than triplicate samples were collected at each location, each sample coming from a different piling.

Sampling Locations

Transplanted mussels were deployed in the Duwamish Waterway at the Brandon Street CSO, at the Duwamish/Diagonal Way CSO/storm drain, at the Chelan Street CSO, and at two in-river reference sites (Figure 1). Separated storm water is also discharged through the Duwamish/Diagonal CSO outfall. Additionally, transplanted mussels were deployed in Elliott Bay at the Denny Way CSO and at a marine reference site (Taylor United, Inc. mussel farm on Totten Inlet). The mussel transplants from the Denny Way and Chelan Street CSOs were lost possibly due to vandalism or storm activity.

The first in-river reference site (Slip #1) was located approximately 500 m below the Brandon Street CSO on the east (same) side of the river. The second in-river reference site was located at the farthest downstream point of Kellogg Island and is approximately 300 m west of the Duwamish/Diagonal CSO/storm drain. Sediments at these sites were either previously sampled as part of the Elliott Bay Action Program (PSEP, 1988) or by King County, and were found not to violate Washington State Sediment Management Standards (Chapter 173-204-WAC) for either metals or organics (R. Shuman, King County Water and Land Resources Division, Seattle, Washington, personal communication).

Wild mussels were collected in the Duwamish Waterway from Slip #4, Brandon Street CSO, Duwamish/Diagonal Way CSO/storm drain, Terminal 105, Hanford Avenue CSO, and in Elliott Bay at the Elliott Bay Pier (Figure 1).

At each CSO location, the mussel transplants were deployed immediately in front of or just below (downriver) the discharge pipe. Distance from the outfall to the mussel transplants was 25 m or less. At these same locations, wild mussels were collected as close to the outfall as practical. Wild mussels were collected approximately 25 m below the Brandon Street CSO outfall, approximately 50 m above the Duwamish/Diagonal CSO/storm drain outfall, and approximately 50 m below the Hanford CSO outfall. The Duwamish/Diagonal Way CSO/storm drain wild mussel collection site is affected by the discharge plume on the incoming tide; that is, the discharge plume bends upriver on the incoming tide.

Salinity and Other Water Quality Parameters

Salinity, temperature, dissolved oxygen, and pH were measured twice at each transplant station during the dry season deployment and once during the wet season deployment. Additionally, these parameters were measured weekly at a subset of the transplant locations (Brandon Street CSO, Chelan Street CSO) and elsewhere (Hanford Avenue CSO) over the greater wet season (December 1996 to June 1997). The latter data are not presented in this paper.

Laboratory Processing

After retrieval, the mussels were shucked and the available tissue composited for chemical analyses. Approximately 110 g of tissue was pooled from the 50 mussels from each bag to represent a single sample. Using clean gloves, dissections were conducted with teflon knives on the frozen tissue tp prevent fluid loss internal to the shell. Tissues were homogenized in a blender fitted with titanium blades. Puget Sound Estuary Program methodology (PSEP, 1996a) was followed for the storage of tissues until chemical analysis.

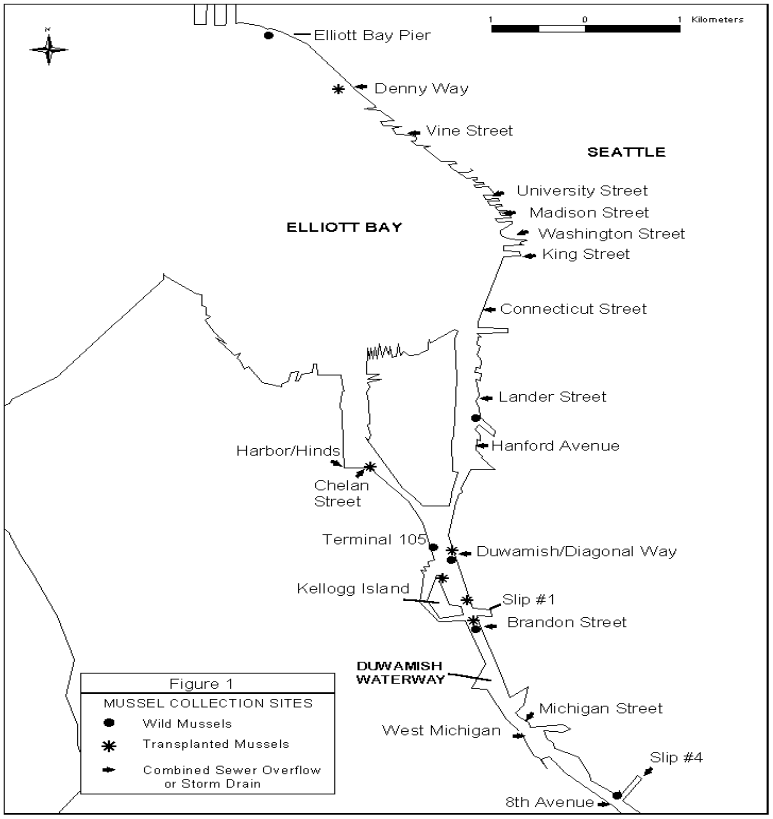

Figure 1. Collection sites for wild mussels in the Duwamish Waterway and Elliott Bay.

Chemical Analytical Procedures

Tissue digests, extractions, and other analytical procedures also followed the Puget Sound Estuary Program methodology (PSEP, 1996b, 1996c). Copper was analyzed by ICPMS (inductively coupled plasma mass spectrometry) following U.S. Environmental Protection Agency (USEPA) Method 6020. PCB analyses were performed following USEPA Method 8082 (SW-846) using GC dual-ECD (gas chromatography dual-electron capture detector) techniques. Tributyltin (TBT) was analyzed by GCFPD (gas chromatography flame photometric detection) following the methods of Unger et al. (1986). Percent lipids were determined gravimetrically.

Statistical Analysis

Non-parametric methods were used at the first level of statistical analysis. For analysis that involved comparison of mussel groups from two locations or depths, the Kolomogorov-Smirnov Statistic was applied (Conover, 1980). For multiple comparisons, the Mann-Whitney Statistic (Winkler and Hayes, 1975) was used. If significant differences were found, an analysis of variance (ANOVA) followed by Bonferroni's Modified Least Significant Difference Test (Timm, 1975) were used to identify specific differences.

Results

Concentrations (medians and interquartile ranges) of the three chemicals and percent lipids in composite samples of transplanted and wild mussels from the Duwamish River estuary are presented in Table 1.

Table 1. Chemical concentrations in composites of 60 mussels.

	[redacted]											
	Median	**IQR [a]**	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	**N**	**Median**	**IQR**	**N**
DRY SEASON												
[redacted]												
Brandon Street-1	1.18	0.04	5	47.3	5.9	5	56.4	5.4	4	0.99	0.51	5
Slip #1-1 (Ref)	1.22	0.11	5	35.6	14.6	5	65	6.7	5	1.2	0.1	5
Duwamish/Diagonal-1	1.14	0.25	5	49	12.7	5	60.6	9.8	4	1.1	0.1	5
Duwamish/Diagonal-3	1.48	0.01	2	42.8	6.7	2	54.8	0.75	2	0.95	0.01	2
[redacted]												
Brandon Street	0.97	0.26	3	37.8	7.8	5	43.3	5.4	3	0.53	0.4	3
Duwamish/Diagonal	1.01	0.04	5	70.3	25.1	5	52.3	10.2	5	0.68	0.42	5
Terminal 105	1.14	0.08	3	59.5	8.1	3	31.2	1.1	3	0.77	0.06	3
Hanford Avenue	1.53	0.17	3	164	41.5	5	28.7	2.1	3	0.73	0.09	3
[redacted]	0.86	0.07	7	3.3	0.4	7	<MDL[b](13)	--	7	2.2	0.35	7
WET SEASON												
[redacted]												
Brandon Street-1	0.58	0.1	3	15.5	2.1	2	<MDL (13)	--	3	0.61	0.25	3
Brandon Street-3	0.87	0.1	3	27.6	6.2	3	<MDL (13)	--	3	1.4	0.38	3
Kellogg Island-1 (Ref)	0.85	0.01	2	9.3	--	1	<MDL (13)	--	2	1.3	--	1
Kellogg Island-3 (Ref)	0.99	0.23	5	18.3	3.7	4	<MDL (13)	--	5	1.74	0.34	3
Duwamish/Diagonal-1	1.07	0.7	4	11.6	1.8	4	<MDL (13)	--	4	1.5	0.82	4
Duwamish/Diagonal-3	1.69	0.43	3	27.5	5.6	3	<MDL (13)	--	3	2.32	0.3	3
[redacted]												
Slip #4	1.18	0.06	3	21.8	1.1	3	53.6	12.3	3	1.05	0.32	3
Brandon Street	1.69	0.11	3	31.3	6.7	3	27.7	1.4	3	1.2	0.25	3
Duwamish/Diagonal	1.31	0.18	3	18.5	5.2	3	<MDL (13)	--	3	0.64	0.21	3
Terminal 105	1.04	0.07	3	19.9	4.2	3	<MDL (13)	--	3	0.46	0.12	3
Hanford Avenue	2.15	0.03	3	72.8	4.7	3	<MDL (13)	--	3	1.07	0.06	3
Myrtle Edwards Park	1.1	0.03	3	58.2	0.5	3	<MDL (13)	--	3	2.3	1.3	3
[redacted]	0.48	0.07	6	3.2	2.5	6	<MDL (13)	--	6	2.7	0.53	6

[a]Interquartile range
[b]Method detection limit

Copper

Copper concentrations in transplanted mussels ranged from 0.58 to 1.69 milligrams/kilogram (mg/kg) wet weight. The highest concentrations were found near the Duwamish/Diagonal Way CSO/storm drain both during the dry season and the wet season. The concentrations in Duwamish/Diagonal Way CSO/storm drain mussels were significantly greater than the concentrations in mussels from other transplant locations ($P<0.05$). The lowest concentrations of copper were found in mussels deployed at the marine reference site. Concentrations of copper were generally lower in transplants deployed during the wet season when compared to transplants deployed during the dry season ($P<0.05$). Also, at the Brandon Street CSO in the wet season, concentrations of copper were higher in mussels deployed near the bottom than mussels deployed near the surface ($P<0.05$).

Copper concentrations in wild mussels ranged between 0.97 and 2.15 mg/kg wet weight. Highest concentrations in wild mussels occurred near the Hanford Avenue CSO sampling site, both during the dry (1.53 mg/kg wet weight) and wet seasons (2.15 mg/kg wet weight). Metals from both a shipyard and an abandoned lead smelter may influence this site. The concentration of copper accumulated at this site is higher than the concentrations of copper accumulated at all other wild mussel collection sites ($P<0.05$). Contrary to what we observed in transplanted mussels, the concentrations of copper found in wild mussels were generally higher during the wet season. This was true at all CSO sampling sites (Brandon Street, Duwamish/Diagonal Way, and Hanford Avenue). These differences are statistically significant at the $P<0.05$ level. The exceptions were Terminal 105 and the marine reference site, which had higher concentrations in the dry season.

We also compared copper concentrations in transplanted mussels with concentrations of copper in wild mussels at the same locations to evaluate the rate of uptake by transplanted mussels over their one-month exposure. We learned that in the dry season the copper concentrations in transplanted mussels from the Brandon Street CSO and Duwamish/Diagonal CSO/storm drain were essentially the same as the concentrations of copper in the wild mussels from these locations. These data suggest that uptake of copper was rapid, reaching equilibrium in one month. The copper concentrations in transplanted mussels in the wet season, however, did not always reach the levels of copper concentrations in the wild mussels. This was true at Brandon Street and also at Kellogg Island. The Kellogg Island transplants were compared to the Terminal 105 wild mussels.

TBT

In the dry season, concentrations of TBT in transplanted mussels ranged between 3.3 micrograms/kilogram (μg/kg) wet weight and 49.0 μg/kg wet weight. The lowest concentrations occurred at the marine reference site, the highest concentrations at Duwamish/Diagonal Way CSO/storm drain. There were no significant differences among in-river transplant locations ($P<0.05$). During the wet season, concentrations of TBT in transplanted mussels were significantly lower ($P<0.05$) ranging between 3.2 and 27.6 μg/kg wet weight. During the wet season, differences in concentration were also apparent with depth. Concentrations of TBT at the Brandon Street CSO were significantly greater ($P<0.1$) in mussels maintained at the –3m MLLW level when compared with mussels maintained at –1m MLLW. At the Duwamish/Diagonal CSO/storm drain, concentrations of TBT in mussels maintained at the –3 m MLLW level were also greater ($P<0.05$) than the concentrations of TBT in mussels from the –1 m MLLW level.

In the dry season, concentrations of TBT in wild mussels were higher at the Duwamish/Diagonal Way CSO/storm drain than at the Brandon Street CSO. They were higher yet at Terminal 105 and highest near the Hanford Avenue CSO. These differences are significant at the $P<0.05$ level. While this gradient is less clear in the wet season, the highest concentrations of TBT were again encountered below the Hanford Avenue CSO. Concentrations of TBT in wild mussels were also significantly lower in the wet season than in the dry season ($P<0.01$). Concentrations in wild mussels from Elliott Bay Pier were less than the concentrations in wild mussels from Duwamish/Diagonal CSO/storm drain but greater than the concentrations in wild mussels from the Hanford Avenue CSO sampling site.

Where data are available for both transplanted and wild mussels at the same site (Brandon Street and Duwamish/Diagonal CSO/storm drain), no statistically significant ($P<0.05$) differences were apparent in concentrations of TBT between transplanted and wild mussels. These data suggest that TBT was rapidly accumulated to an equilibrium level.

PCBs

While PCBs were not detected in transplanted mussels maintained at the marine reference Site, they were detected in all transplants in the Duwamish Waterway in the dry season. Concentrations ranged between 54.8 μg/kg wet weight and 65.0 μg/kg wet weight. PCB concentrations in wild mussels in the dry season generally appeared to be lower than PCB concentrations in transplanted mussels in the dry season, if compared on a wet weight basis. If normalized to percent lipid (data not shown), however, the concentrations of PCBs among all locations (transplant and wild) in the dry season were not statistically different (P<0.05), suggesting that accumulation in transplanted mussels occurred rapidly, reaching equilibrium over the four-week deployment. PCBs were only found in wild mussels in the wet season at Slip #4 (53.6 μg/kg wet weight) and at Brandon Street CSO (27.7 μg/kg wet weight).

Lipids

Lipid content was generally highest in transplanted mussels maintained at the marine reference site. Values were 2.20% in the dry season and 2.70% in wet season. For transplants deployed in the Duwamish Waterway in the dry season, lipid content varied between 0.99% and 1.20%. Lipid content in transplants tended to be higher in the wet season, ranging between 0.61% and 2.30%. Lipid content also tended to vary with depth. Mussels maintained at –3 m at the Duwamish/Diagonal CSO/storm drain in the wet season contained more lipid than mussels maintained at –1 m (P<0.05). Although not shown in Table 1, lipid contents of mussels deployed at the marine reference site did not change from their pre-deployment levels, both in the dry and in the wet seasons.

Wild mussel lipid content in the dry season varied over the range of 0.53% to 0.77%. The range in the wet season was generally higher, 0.46% to 2.32%. The difference is statistically significant (P<0.05) for most locations where comparative data were available. The highest value (2.32%) was found in mussels from the Elliott Bay Pier in the wet season.

Salinity Regime and Other Water Quality Parameters

Salinity varies with tidal stage and runoff. During the dry season in the Duwamish Waterway, salinity tends to be greater. For example, at the ebb for the first low tide of the day at Brandon Street CSO on October 8, 1996, salinity was 22.6 parts per thousand (ppt) at –1m MLLW and 27.1 ppt at the –3m MLLW level. The river flow on this date was 238 cubic feet per second (cfs). The temperature, dissolved oxygen, and pH at the –1 m MLLW level were 13.0 _C, 6.1 parts per million (ppm), and 7.0, respectively. At the –3 m MLLW level , these parameters were 12.6 _C, 5.7 ppm, and 7.12, respectively. During the wet season, salinity in the Duwamish Waterway decreases significantly as runoff and river flows increase. On March 26, 1997, at the ebb of the first low tide of the day at Brandon Street CSO, salinity at –1m MLLW was only 3.0 ppt. Salinity at –3m MLLW was 8.0 ppt. River flow was 3,080 cfs. The temperature, dissolved oxygen, and pH at –1 m MLLW were 8.7 _C, 10.5 ppm, and 7.2, respectively. These parameters were not greatly different at –3 m MLLW.

Discussion

Historical Data

Several previous studies report historical levels of copper, PCBs, and TBT in mussels from the Duwamish River estuary. Copper concentrations in mussels from Four-Mile Rock (Elliott Bay) in 1986, 1987, and 1988, were 12, 8.7, and 11 μg/g dry weight, respectively (NOAA, 1989). These convert from dry weight to wet weight (assuming 87% moisture content [Johnson and Davis, 1996]) to 1.56, 1.13, and 1.43 mg/kg wet weight, respectively. These concentrations agree well with the values (0.97–1.53 mg/kg wet weight) reported here.

NOAA (1989) also analyzed the mussels from Elliott Bay for total PCBs and found concentrations of 1110, 580, and 450 μg/kg dry weight for 1986, 1987, and 1988, respectively. Converting from dry weight to wet weight, these concentrations become 143, 75, and 58 μg/kg wet weight. NOAA's 1988 total PCB concentration is relatively close to concentrations (54.6–65.0 μg/kg wet weight) observed in the present study. Johnson and Davis (1996) found 44 μg/kg wet weight of PCBs in mussels from the lower Duwamish Waterway, which also provides excellent agreement with the level (28.7 μg/kg wet weight) found in the lower Duwamish Waterway (Hanford Avenue CSO) in this study.

The TBT data in mussels from the Duwamish Waterway presented in Table 1 are the first such data from the waterway to be published. These data indicate that highest levels occurred in the lower waterway where shipping and ship repair activities are concentrated. Short and Sharp (1989) included three locations in Elliott Bay in their 1986 and 1987 surveys. They found concentrations ranging from 50 to 230 μg/kg wet weight, which are not greatly different from the range of values (35.6 and 164.0 μg/kg wet weight) reported in the dry season in this study.

While numerous measurements of lipid content for mussels have been published, perhaps the most germane to the present study are the results of Johnson and Davis (1996). They determined that wild mussels from the Duwamish Waterway contained 1.1% lipid, which is in the range of values for wild mussels (0.53%–2.3%) reported in this study.

Dynamics of Chemical Exposure

We have interpreted our results to mean that exposure in the Duwamish Waterway changes seasonally. Based on the transplanted mussel data, exposure to copper, TBT and PCBs tended to decrease during the wet season throughout the study area. Based on wild mussel data, however, exposure to copper may have increased in the wet season at some locations.

The findings that copper, TBT, and PCBs in transplanted mussels decreased in the wet season first suggested an alternative explanation. Knowing that both transplanted and wild mussel types were exposed to lower temperatures and very low salinities during the wet season, we first thought that the mussels had stopped pumping/feeding and that they had lived off their lipid reserves over this period. They then might not be expected to accumulate as much chemical. This may not be the case because lipid levels remained quite high in the transplanted mussels throughout the wet season deployment. Actually, lipid levels for both transplanted and wild mussels were generally higher during the wet season when compared with the dry season. Also, more copper was accumulated by the wild mussels near CSOs during the wet season suggesting that the mussels continued to pump and feed normally.

Sources of Bioaccumulatable Copper, TBT, and PCBs in the Duwamish Estuary

At all CSO locations, the concentrations of copper were found to increase in wild mussels in the wet season. This increase in tissue concentration was 29.7%, 40.5%, and 74.2% at the Brandon Street CSO, Duwamish/Diagonal CSO/storm drain, and Hanford Avenue CSO, respectively. One possible source of this copper is the CSOs/storm drains. Copper (mean of 43.6 μg/L) is known to occur in the stormwater component of CSOs (e.g., Hinds CSO) discharging to the Duwamish Waterway and has been found at 41.1–119.0 mg/kg dry weight in the sediments from the Duwamish/Diagonal storm drain (King County, 1997a). Copper commonly occurs in CSOs discharging elsewhere to Puget Sound, e.g., City of Bremerton (Fohn 1997). While the data are not presented in this paper, the concentrations of cadmium, lead, and zinc also increased in wild mussels collected near the Brandon Street CSO, Duwamish/Diagonal CSO/storm drain, and Hanford Avenue CSO in the wet season.

Another possible source of the bioavailable copper found in wild mussels near the CSOs is the sediments. It is conceivable that copper deposited to the sediments from past overflows could have been resuspended during recent (winter 1996–1997) overflows, although we might have expected other chemicals, e.g., PCBs, to be resuspended and accumulated in wild mussels at higher concentrations in the wet season, which was not the case. Both the Brandon Street and Duwamish/Diagonal CSOs discharge directly to the intertdial zone and have the potential to scour chemicals from the surface sediments. Copper concentrations in the sediments at the Brandon Street CSO range between 61.0–72.4 mg/kg dry weight. They range between 62.9 and 108 mg/kg dry weight in the sediments near the Duwamish/Diagonal CSO and storm drain.

Ground water is another possible source of the increased copper accumulation in the wild mussels. Ground water collected from wells on the Chiyoda/Chevron property, which is just upriver from the

Duwamish/Diagonal Way outfall contained a maximum of 200 μg/l copper (King County 1997). There are no comparable analyses of groundwater sources near the Brandon Street or Hanford Avenue CSOs.

The fact that the increase in copper in wild mussels cannot be explained by normal seasonal differences in copper concentrations in the Duwamish Waterway is supported by the finding that we did not find an increase in copper in wild mussels from the Terminal 105 site. The Terminal 105 site is on the opposite (east) side of the river from where most CSOs and storm drains discharge.

The fact that the transplanted mussels were not exposed to the same number of overflows and storm drain discharges as the wild mussels might explain why the transplanted mussels failed to demonstrate a commensurate increase in copper concentrations at the three CSO locations. The transplanted mussels were only exposed to six overflows at Brandon over the one-month deployment period, and only one overflow at the Duwamish/Diagonal Way CSO/storm drain over this period. The wild mussels were exposed to many more overflows since the beginning of the wet season in October 1996.

Additional work is underway to address the source of copper and other metals found to increase in some wild mussel tissues during the wet season. A hydrodynamic and chemical fate mathematical model presently being developed by King County may be particularly helpful in this regard (King County, 1997b).

We also believe that the findings of less TBT and almost no PCBs in both transplanted and wild mussels in the wet season reflect different sources of these chemicals in the estuary. Because the source of PCBs in the Duwamish Waterway is the sediments, we might not expect mussels to bioaccumulate much PCB if the overlying and less-dense lens of fresh water occurring in the wet season acts as a barrier to the complete mixing of PCBs into the water column from the sediments.

While TBT also partitions to the water column from the sediments and would be subjected to the same hydrodynamic processes as PCBs, TBT is also known to elute into the water column from the hull paints/coatings of ships at moorage in the Duwamish Waterway and Elliott Bay. This is a direct input of TBT to the overlying lens of fresh water entering the waterway, which could account for more TBT becoming available for bioaccumulation. While less TBT was found in mussels in the wet season, there was not the near-total exclusion observed for PCBs. Also, more TBT in the mussel transplants maintained at the –3-m depth in the wet season tends to support this interpretation.

Conclusions

1) Bioaccumulation varied with both location in the estuary and with depth. Concentrations of copper and TBT in wild mussels were highest in the lower Duwamish Waterway while concentrations of PCBs tended to be more uniform over the study area. In the wet season, mussel transplants indicated that concentrations of copper and TBT were greater at –3 m than at –1 m MLLW.
2) Bioaccumulation also varied seasonally in the Duwamish Waterway. Concentrations of all three chemicals were lower in mussel transplants in the wet season. This may be due to freshwater runoff. This was particularly true for TBT and PCBs whose sources may not be dependent on wet weather events. Wet season levels of copper in wild mussels were higher at some locations. Additional work is underway to determine the source of increased levels of copper and other metals found in some wild mussels in the wet season.

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